Cell, Vol. 118, 671–674, September 17, 2004, Copyright ©2004 by Cell Press

At the Crossroads of Inflammation and Cancer

Minireview

Hans Clevers*
Hubrecht Laboratory
Center for Biomedical Genetics
Uppsalalaan 8
Utrecht 3584 CT
The Netherlands

Chronic inflammation and cancer are closely associated in the intestine. Anti-inflammatory medication reduces intestinal neoplasia, while colorectal cancer incidence is increased in ulcerative colitis. Cyclooxygenases are key to both diseases, yet the molecular basis of the association remains incompletely understood. Two recent *Cell* (Greten et al., 2004; Rakoff-Nahoum et al., 2004) papers illuminate roles of Toll-like receptors and the NF-κB pathway in the control of epithelial homeostasis in health and disease.

Ulcerative colitis (UC) is a common chronic inflammatory disease of unknown etiology, which affects the mucosa of the large bowel. UC is associated with an elevated risk for colorectal cancer, which is in the order of 10-fold greater than in the general Western population (reviewed in Itzkowitz and Yio, 2004; Seril et al., 2003). The risk of developing cancer increases strongly with the duration and the extent of the disease. Indeed, UC ranks among the top three high-risk conditions for colorectal cancer, together with the Familial Adenomatous Polyposis (FAP) and Hereditary Nonpolyposis Colorectal Cancer (HNPCC) syndromes. Unlike the latter two conditions that have a well-understood genetic etiology, it is unlikely that the association of UC with colorectal cancer results from an underlying genetic cancer predisposition. Rather, it is now commonly believed that it is the chronic inflammation process itself that is responsible for the occurrence of neoplastic transformation of the intestinal epithelium (Itzkowitz and Yio, 2004). An additional link exists between inflammation and colorectal cancer. Epidemiological studies have indicated that the regular administration of nonsteroidal anti-inflammatory drugs (NSAIDs) lowers mortality from sporadic colorectal cancer and causes regression of adenomas in patients with FAP (reviewed in Oshima and Taketo, 2002). The specific molecular mechanism behind this phenomenon remains unknown.

Sporadic colorectal cancer is probably the best understood solid malignancy in man. Histologically, it progresses in a stepwise fashion, termed the adenoma-carcinoma sequence. As originally proposed by Fearon and Vogelstein (1990), defined molecular alterations underlie these progressive changes in histology. Typically, the neoplastic lesions first present as aberrant crypt foci or microadenomas and develop through a large adenoma-stage into carcinoma in situ and invasive adenocarcinoma. When UC-associated colorectal cancer is compared to its sporadic counterpart, it is immediately clear that it follows a different histological sequence (Seril et al., 2003; Itzkowitz and Yio, 2004), starting in the inflamed mucosa as a hyperplastic lesion, to develop through (flat) dysplasia into adenocarcinoma. This is sometimes summarized as the "inflammation-dysplasia-carcinoma" sequence. Although these differences may suggest divergent pathogenic pathways, molecular studies indicate that the transformation process is largely shared between sporadic and UC-associated colorectal cancer. The relative order in which mutations in genes such as APC, P53, SMAD4, or K-ras may differ, yet these mutations are observed with comparable frequencies in both disease entities. It thus appears reasonable to consider the two forms of colorectal cancer, once initiated, as a single disease entity.

Multiple animal models have been established for the dissection of the effects of inflammatory mediators and anti-inflammatory drugs on UC-associated, hereditary, and sporadic forms of colorectal cancer (for an exhaustive overview, see Oshima and Taketo, 2002; Koehne and Dubois, 2004). It is important to note that these models are somewhat limited in their interpretation of the human condition, since the cause of UC remains unknown. Roughly, the models can be divided into two groups, one in which colitis is induced by chemical means and one in which colitis develops due to genetic manipulation (reviewed in Seril et al., 2003; Itzkowitz and Yio, 2004). The most widely used chemical induction model involves the administration of dextran sulfate sodium (DSS) in the diet of rodents. DSS is a synthetic, sulfated polysaccharide that causes acute and chronic colitis (reviewed in Seril et al., 2003). Carcinomas with involvement of the APC pathway are reportedly induced by DSS in rats, hamsters, and mice. DSS treatment of the APC mutant *min* mouse as well as the combination of DSS treatment with a carcinogen, such as azoxymethane (AOM), represent extremely efficient ways to generate adenocarcinomas of the intestine. In most chemical models, chronic inflammation by disruption of the mucosal barrier function and the concomitant immune hyperactivation by the microflora is presumed to represent the key event that creates a microenvironment that is permissive for the progressive transformation of colon epithelial cells. The genetic models commonly affect genes that regulate the immune response (Seril et al. 2003), creating a state of chronic hyperactivity of the adaptive immune system. As with the chemically induced colitis models, efficient development of colitis requires the mucosa-associated microflora, as demonstrated by comparing mice raised under specific-pathogen-free and germfree conditions (Schuppler et al., 2004 and references therein).

The prevailing view on the association of UC and cancer involves an indirect mechanism, in which inflammation of the submucosa, induced by direct contact with the intestinal microflora, promotes tumor outgrowth in the overlying epithelium. Two recent papers in *Cell* (Greten et al., 2004; Rakoff-Nahoum et al., 2004) provide support for an alternative model in which inflammatory

*Correspondence: clevers@niob.knaw.nl

signaling in the epithelial cells proper results in their inappropriate survival and transformation. Activated NF-κB, the key regulator of inflammation, is present in many solid tumors and cell lines derived thereof (reviewed in Amit and Ben-Neriah, 2003). Noting this, Karin and colleagues (Greten et al., 2004) set out to study the role of the NF-κB pathway in a mouse model for UC-associated colorectal cancer. To this end, they deleted an essential component of the pathway, the IKKβ kinase, either in all myeloid cells or in the intestinal epithelial cells. Disruption of the NF-κB pathway in myeloid cells essentially confirmed a scenario by which inflammation creates a signaling niche in which transformed cells prosper. When the genetically modified mice were subjected to a chemically induced colitis/carcinogen protocol (DSS/AOM, see below), the numbers and size of the adenomas were substantially reduced. As expected, induction of inflammatory mediators including COX-2 was substantially decreased. The authors concluded that the lower tumor incidence was due to the loss of production of tumor-promoting paracrine factors.

The unexpected result came when the NF-κB pathway was deleted in intestinal epithelial cells. In a previous study, the authors had found that IKKβ removal from these cells did not affect normal homeostasis of the epithelium. However, the mutant epithelium was found to be much more sensitive to ischemia-reperfusion-induced apoptosis, implying a cell-autonomous role for the NF-κB pathway in epithelial cell survival (Chen et al., 2003). Accordingly, the mutant epithelium appeared more susceptible to DSS-induced histological damage than control epithelium, while the production of many inflammatory mediators was actually enhanced. Despite this, the colitis-associated tumor incidence in the DSS/AOM model was greatly reduced. The authors then went on to show that an active NF-κB pathway contributes to tumor formation by providing antiapoptotic survival signals to the epithelial cells. While the exact molecular mechanism of this effect remains to be detailed, the study suggests that IKKβ acts through suppression of the mitochondrial apoptosis pathway by induction of the NF-κB target gene Bcl-x_L.

What could be the nature of the signal that triggers this epithelial NF-κB survival pathway? Rakoff-Nahoum et al. (2004) may provide a clue by showing that commensal bacteria can activate a "cell-survival" pathway downstream of Toll-like receptors (TLRs). It had long been known that the resident microflora of the intestinal tract confers many benefits to intestinal physiology, including trophic effects on the epithelium (Hooper and Gordon, 2001 and references therein). Medzhitov and colleagues wondered whether Toll-like receptors (TLRs) that play a crucial role in host-defense could respond to commensal bacteria. TLRs are pattern-recognition receptors that detect common microbial ligands. Prototypic examples of these ligands are lipopolysaccharides and lipoteichoic acid (LTA), recognized by TLR4 and TLR2 respectively (Takedo et al., 2003). Curiously, pathogenic as well as commensal bacteria produce these ligands.

Rakoff-Nahoum et al. (2004) used mice deficient in TLR signaling due to a deletion of the central adaptor protein MyD88. Alternatively, they used mice lacking

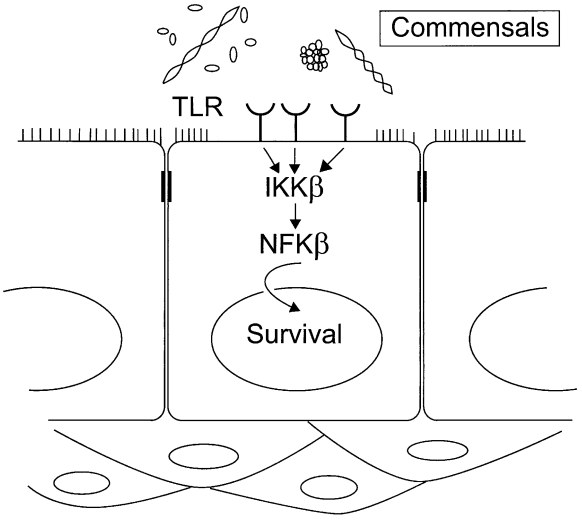

Figure 1. Commensals Provide Survival Signals through TLR Receptors

The commensal intestinal flora activates Toll-like Receptors (TLR) on the luminal surface of intestinal epithelial cells (Rakoff-Nahoum et al., 2004). This interaction triggers the activity of intracellular signaling pathways, such as the IKK-β/NF-κB cascade, providing potent survival signals for the epithelial cell (Greten et al., 2004). Figure courtesy of Geert van Eldik.

TLR2 or TLR4. In all cases, this unexpectedly led to increased susceptibility to DSS-induced colonic injury. The authors went on to demonstrate that—under physiological conditions—commensal bacteria activate TLRs, and that the resulting TLR activity provides protection from colitis-induced damage. Since the authors did not apply cell type-specific gene ablation, it remains to be formally demonstrated in which cell type TLR signaling normally acts to enhance epithelial integrity. Yet, the data fit best with a model in which recognition of bacterial products occurs by TLRs expressed on enterocytes, residing in an intact epithelial sheet. These activated TLRs would then mediate a cell-autonomous cell-survival response. Of note, the major signaling pathway downstream of TLRs and MyD88, conserved from fly to man, is the NF-κB pathway (Beutler, 2004). The data of the recent Karin and Medzhitov studies can be integrated into a simple model (Figure 1) in which the commensal flora releases TLR ligands, e.g., lipopolysaccharides. These ligands trigger the TLR/MyD88/ NF-κB pathway, providing survival signals to the enterocyte.

Cell-Autonomous Effects of Inflammation Pathways in Intestinal Epithelium?

The previously published literature overwhelmingly supports a scenario in which inflammation promotes (rather than initiates) carcinogenesis in a non-cell-autonomous fashion. Loss of epithelial barrier function would lead to direct contact between the intestinal microflora and the immune system. The resulting activity of inflammatory mediators, such as COX-2, would then create a tumor-promoting environment in which transformed epithelial cells thrive more optimally. Strong genetic support for this indirect model comes from observations made in mice mutant for the major targets of NSAIDs, i.e., the

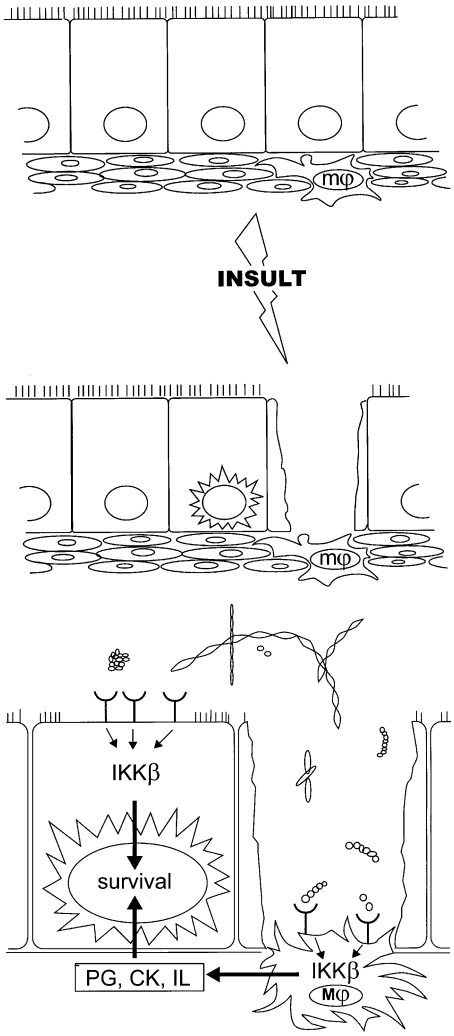

Figure 2. Two NFκB Pathways Promote Tumorigenesis

Physical, chemical, or microbial insults cause mutations (transforming epithelial cells), and break epithelial integrity (exposing underlying cell layers). The intestinal flora triggers the NF-κB pathway in macrophages to release proinflammatory agents such as prostaglandins (PG), chemokines (CK), and interleukins (IL) that indirectly promote survival of transformed epithelial cells. Independently, the intestinal flora directly triggers the survival NF-κB pathway through TLRs on the transformed cell. Figure courtesy of Geert van Eldik.

COX-1 and -2 enzymes. As expected, adenoma formation in APC mutant intestine was strongly reduced in both the COX1$^{-/-}$ and the COX-2$^{-/-}$ background (Oshima and Taketo, 1996). Involvement of the products of the COX biochemical pathway, i.e., prostaglandins, in adenoma formation of APC mutant mice was deduced from the dramatic reduction of adenomas in compound mutants with the prostaglandin receptor EP2$^{-/-}$, but not EP1 or EP3 (Sonoshita et al., 2001).

Against this backdrop, the two papers in *Cell* (Rakoff-Nahoum et al., 2004; Greten et al., 2004) provide evidence for an alternative, direct involvement of inflammatory signaling in survival and transformation of the epithelial cell proper. A dual mechanism can now be proposed by which commensal bacteria contribute to UC-associated colorectal cancer (Figure 2). Commensal bacteria constitutively activate the NF-κB pathway through TLRs expressed on epithelial cells, supporting cell survival and protecting the epithelium from injury. When stimulated in transformed epithelial cells, this pathway accelerates tumor development in a cell-autonomous fashion. This mechanism complements the non-cell-autonomous inflammation scenario, in which mediators produced by inflamed stroma promote tumorigenesis. Moreover, the existence of the cell-autonomous pathway may also explain the beneficial effects that are seen with anti-inflammatory treatment of sporadic colorectal cancer, in which no overt inflammation appears present. If confirmed, future therapeutic strategies for colorectal cancer may take advantage of this cell-autonomous cell-survival role of TLRs and the NF-κB pathway in intestinal epithelium.

The studies raise some immediate questions. TLR signaling by commensal bacteria appears to exert protective effects on the epithelium, while TLR signaling initiated by pathogens leads to inflammation. How are these two broad categories of microorganisms distinguished by the TLR pattern recognition receptors? One possibility may be that the cell type that recognizes the TLR ligands determines the outcome of the interaction. TLR signaling in intact epithelial cells would thus lead to the previously observed trophic response of the epithelial sheet to commensals. On the other hand, direct interaction of bacterial products with submucosal inflammatory cells would only occur when the epithelium is damaged and would thus be associated with the presence of pathogenic bacteria. Another question that can now be answered relates to the identity of the target(s) responsible for the protective effects of NSAIDs in colorectal cancer. With the novel information discussed here, it is likely that the transformed epithelial cell, and not the stromal environment, represents the direct target for anti-inflammatory drug treatment. From this perspective, it is intriguing that several NSAIDs have been demonstrated to inhibit the kinase activity of IKKβ (Yin et al., 1998; Yamamoto et al., 1999)

Selected Reading

Amit, S., and Ben-Neriah, Y. (2003). Semin. Cancer Biol. *13*, 15–28.

Beutler, B. (2004). Nature *430*, 257–263.

Chen, L.W., Egan, L., Li, Z.W., Greten, F.R., Kagnoff, M.F., and Karin, M. (2003). Nat. Med. *9*, 575–581.

Fearon, E.R., and Vogelstein, B. (1990). Cell *61*, 759–767.

Greten, F.R., Eckmann, L., Greten, T.F., Park, J., Li, Z., Egan, L.J., Kagnoff, M.F., and Karin, M. (2004). Cell *118*, 285–296.

Gupta, A.G., and DuBois, R.N. (2001). Nat. Rev. Cancer *1*, 11–21.

Hooper, L.V., and Gordon, J.I. (2001). Science *292*, 1115–1118.

Itzkowitz, S.H., and Yio, X. (2004). Am. J. Physiol. Gastrointest. Liver Physiol. *287*, G7–G17.

Koehne, C.H., and Dubois, R.N. (2004). Semin. Oncol. *31 (2 Suppl. 7)*, 12–21.

Oshima, M., and Taketo, M.M. (2002). Curr. Pharm. Des. *8*, 1021–1034.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., and Medzhitov, R. (2004). Cell *118*, 229–241.

Rogers, A.B., and Fox, J.G. (2004). Am. J. Physiol. Gastrointest. Liver Physiol. *286*, G361–G366.

Seril, D.N., Lia, J., Yang, G.Y., and Yang, C.S. (2003). Carcinogenesis *24*, 353–362.

Schuppler, M., Lotzsch, K., Waidmann, M., and Autenrieth, I.B. (2004). Infect. Immun. *72*, 1983–1990.

Sonoshita, M., Takaku, K., Sasaki, N., Sugimoto, Y., Ushikubi, F., Narumiya, S., Oshima, M., and Taketo, M.M. (2001). Nat. Med. *7*, 1048–1051.

Takeda, H., Sonoshita, M., Oshima, H., Sugihara, K., Chulada, P.C., Langenbach, R., Oshima, M., and Taketo, M.M. (2003). Cancer Res. *63*, 4872–4877.

Takedo, K., Kaisho, T., and Akira, S. (2003). Annu. Rev. Immunol. *21*, 335–376.

Yamamoto, Y., Yin, M.J., Lin, K.M., and Gaynor, R.B. (1999). J. Biol. Chem. *274*, 27307–27314.

Yin, M.J., Yamamoto, Y., and Gaynor, R.B. (1998). Nature *396*, 77–80.

Note Added in Proof

The role of the NF-κB pathway in linking chronic inflammation and cancer is not restricted to the gut. In an online paper in Nature ("NF-B functions as a tumour promoter in inflammation-associated cancer" Pikarsky, E., Porat, R.M., Stein, I., Abramovitch, R., Amit, S., Kasem, S., Gutkovich-Pyest, E., Urieli-Shoval, S., Galun, E., and Ben-Neriah, Y., published online August 25, 2004 doi:10.1038/nature02924), Ben-Neriah and colleagues study MDR-2 knockout mice as a model for chronic hepatitis-associated hepatocellular carcinoma. Using a transgenic approach, the authors demonstrate that hepatocytes require an intact NF-κB pathway to be protected against apoptosis and to progress to hepatocellular carcinoma in a chronic inflammation setting.